

56. (New) The method according to claim 55 wherein said first selection marker and said second selection marker are different antibiotic resistance genes.

57. (New) The method according to claim 55 wherein the adenylate kinase includes mutations at amino acids 87 and 107 in the sequence of *E. coli* adenylate kinase.--

### **REMARKS**

Reconsideration is requested.

Claims 33-46 have been canceled, without prejudice. Claims 47-57 have been added, to advance prosecution and without prejudice. Support for the amended claims may be found throughout the specification. No new matter has been added. Specifically, the claims have been amended in response to the Examiner's comments on page 5 of the Office Action dated July 2, 2002 (Paper No. 19). The Examiner indicated on page 5 of Paper No. 19 that the presently claimed invention is supported by an enabling disclosure. Accordingly, entry of the above amendments will, at a minimum, reduce the issues for appeal by obviating the Section 112, first paragraph, rejection of claims 33-36 and 37-46 stated in paragraph 8 of Paper No. 19.

Entry of the above amendments is requested.

A new Declaration is attached along with a Rule 182 Petition to change the Patent Office records with regard to the last name of the inventor Rachel L. Price which is now Rachel L. Leslie. The requisite Petition fee, required by Rule 17(h), is also attached. Acknowledgement of receipt of the attached Petition and new Declaration and grant of the Petition are requested in the Examiner's next Communication.

The objection to claim 34 stated in paragraph 3 of Paper No. 19 will be moot upon entry of the above amendments.

The Section 112, second paragraph, rejection of claims 33-46 stated in paragraphs 4-6 of Paper No. 19 will be moot upon entry of the above amendments. The Examiner's comments and concerns have been considered in drafting the revised claims. The objected-to terms and phrases have not been included in the amended claims.

Entry of the above is requested.

The Section 112, first paragraph, rejection of claims 33-36 and 37-46 will be moot upon entry of the above amendments. The amended claims are submitted to be supported by an adequate written description. The applicants submit that the Examiner's reliance on the CAFC decision in *UC California v. Eli Lilly* to suggest that the adenylate kinase should be characterized by structural information is inappropriate. The Examiner is urged to appreciate that the presently claimed invention provides a recombinant organism and methods of using the same which, in light of the present specification, will be recognized by one of ordinary skill in the art to have been within the applicants' possession as of the time that the application was filed. The Examiner is particularly requested to appreciate that claims 49 and 57 recite the specifically disclosed mutations.

The claims are submitted to be supported by an adequate written description.

The Section 103 rejection of claims 33-46 over EP 373962 in view of Belinga (Journal of Chromatography A 695:33-40), Gilles (PNAS 83:5798-5802), and Kajiyama (Biochemistry 32:13795-13799) will be moot upon entry of the above amendments. The

claimed invention is believed to be patentable over the combination of cited art and consideration of the following remarks in this regard is requested.

The Examiner's principal reference EP 373962 describes the production of proteins which intrinsically have a very high thermostability, in a mesophilic cell. Mesophilic cells are defined in column 2 lines 45-49 as cells which can produce "the desired thermophilic enzyme and whose proteins generally are denatured at a temperature that does not denature the desired thermophilic enzyme". It is clear therefore that the authors envisage the use of temperatures where substantially all the proteins of the host cell are denatured. Suitable mesophilic cells are listed in column 4 and include *E. coli*.

This is possible in the case of the enzymes being produced in EP 0373962 since they are not only thermostable but also thermophilic enzymes, derived from the organisms which live naturally in very high temperature environments and therefore stable at temperatures of 65-100°C as set out in column 3 line 13. Indeed the examples provided show that the cells must be treated at 70°C or even 80°C in order to achieve the desired results.

Luciferase, even thermostable luciferase, is not a thermophilic enzyme of this type. Kajiyama, et al. describe a thermostable luciferase, but admit that this is completely inactivated at 65°C (see column 4 lines 62-63 of the corresponding US Patent No. 5,229,285). Therefore, it is not believed that a skilled person would have considered trying to make luciferase using the system of EP 0373962.

There is nothing in EP 0373962 which would have suggested that the step of removing the contaminants could be effected at temperatures down to as low as 37°C,

as specified in the claims of the present application. Neither would it have been obvious to carry out such a process at these temperatures because the document fails to teach or suggest "mesophilic cells" fulfilling the requirement that their proteins generally are denatured at this temperature.

The mutant *E. coli* of Gilles is thermosensitive at 40°C, but the thermosensitivity is ascribed specifically to a mutation in the adenylate kinase gene. It must be assumed therefore that all other proteins within the mutant retain the usual thermostability. As a result, it is not seen that a reader would have considered the mutant of Gilles a "mesophilic cell" suitable for use in a low temperature version of the method of EP 0373962.

Furthermore, there is no teaching regarding the sensitivity of even this enzyme at 37°C. Therefore there is no teaching or suggestion in this reference which would have made the subject matter of claim 38, for example, obvious.

Belinga teaches a conventional chromatographic method for removing a range of enzymes from luciferase preparations. Adenylate kinase enzyme is only one such enzyme listed as being an "interfering enzyme". It does not rectify the deficiencies of the other references.

Therefore in summary, even if the ordinarily skilled person were to read all the references cited by the Examiner together, which the applicants believe would be unlikely in the absence of impermissible use of hindsight, the claimed invention of the present application would still not have been obvious.

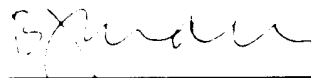
The claims, as amended, are submitted in condition for allowance and Notice to that affect is requested.

SQUIRRELL et al.  
Serial No. 09/529,722

Respectfully submitted,

**NIXON & VANDERHYE P.C.**

By: \_\_\_\_\_



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